

EPA/OPP MICROBIOLOGY LABORATORY  
ESC, Ft. Meade, MD

Standard Operating Procedure  
for  
Testing of Spray Disinfectants Against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Mycobacterium bovis* (BCG)

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### 1.0 SCOPE AND APPLICATION:

- 1.1 This SOP describes the AOAC method to determine the efficacy of spray products as hard surface disinfectants against three test organisms, *Mycobacterium bovis* (BCG), *Pseudomonas aeruginosa*, and *Staphylococcus aureus*.

## 2.0 DEFINITIONS:

- 2.1 AOAC = AOAC INTERNATIONAL
- 2.2 API = Analytical Profile Index
- 2.3 TSA = Trypticase Soy Agar
- 2.4 Carrier Set for *S. aureus* or *P. aeruginosa* = The primary and secondary subculture tubes for each carrier represent a carrier set. There are 60 slide carrier sets per product sample tested.
- 2.5 Carrier Set for *M. bovis* (BCG) = The primary MPB tube containing the slide along with duplicate tubes of two additional subculture media (5 tubes per carrier) seeded from the corresponding neutralizer tube. There are 10 slide carrier sets (5 tubes per set).
- 2.6 Free Water = Water (condensation) that is created during the cooling process after autoclaving capped glassware. This can be avoided by allowing the glassware to slowly cool in the autoclave overnight.

## 3.0 HEALTH AND SAFETY:

- 3.1 All manipulations of the test organism are required to be performed in accordance to biosafety practices stipulated in SOP MB-01, Lab Biosafety.
- 3.2 Disinfectants may contain a number of different active ingredients, such as heavy metals, aldehydes, peroxides, phenol, etc. Latex gloves and other personal protective clothing or devices are worn during the handling of these items for purpose of activation or dilution, or efficacy testing. A chemical fume hood or other containment equipment are employed when performing tasks with concentrated products.

## 4.0 CAUTIONS:

- 4.1 Strict adherence to the protocol is necessary for the validity of the test results.
- 4.2 Do not allow the inoculum to contact the edge of the glass slide carriers during the seeding process. Contamination of the sides of the carriers with the test microbe may lead to false positives.
- 4.3 The external surface of the Eppendorf Pipette used to seed the glass slide carriers with the test organisms may be contaminated during the seeding process. Thus, after completion of the inoculation of the glass slide carriers, the Eppendorf Pipette will be thoroughly wiped with 70% ethanol prior to removal from the BSC.

#### 5.0 INTERFERENCES:

- 5.1 It is important that no free water is present inside the petri dish or on the glass slide carrier prior to testing. Free water inside the petri dish can interfere with the drying process of the inoculum. Free water on the glass slide carrier can interfere with the concentration of the inoculum as well as the spreading and drying of the inoculum on the glass slide.

#### 6.0 PERSONNEL QUALIFICATIONS:

- 6.1 Personnel are required to be knowledgeable of the procedures in this SOP. Documentation of training and familiarization with this SOP can be found in the training file for each employee.

#### 7.0 SPECIAL APPARATUS AND MATERIALS:

- 7.1 *Mycobacterium bovis* BCG (received from Organon Teknika)
- 7.2 *Pseudomonas aeruginosa* (ATCC #15442) (received ATCC)
- 7.3 *Staphylococcus aureus* (ATCC #6538)(received ATCC)
- 7.4 25 × 25 mm glass slides (carriers)
- 7.5 Spray Disinfectant Apparatus - See Attachment B

- 7.6 38 x 100 medication tubes (Bellco) for neutralization and subculture media
- 7.7 VITEK 32 System for the automated identification of microorganisms
- 7.8 Eppendorf Pipettes (1 - 10 uL)
- 7.9 PCS 2 Pipette Calibration System for the calibration of Eppendorf pipettes
- 7.10 Spectrophotometer (Milton Roy Spectronic 20 D)
- 7.11 Spectrophotometer (Beckman DU Series 500)
- 8.0 INSTRUMENT OR METHOD CALIBRATION:
  - 8.1 Refer to the instructions stipulated in SOP QC-19, Calibration of Eppendorf Pipettes, for instrument calibration of the PCS 2 Pipette Calibration System.
- 9.0 SAMPLE HANDLING AND STORAGE:
  - 9.1 Disinfectants are stored according to the manufacturer's recommendations if stipulated, or at room temperature. Those disinfectants requiring activation or dilution prior to use are activated or diluted within three hours of testing unless test parameter specify otherwise.
  - 9.2 Follow chain-of custody guidelines during testing as stipulated in SOP COC-01, Chain-of-Custody.
- 10.0 PROCEDURE AND ANALYSIS:
  - 10.1 Brief Summary: The AOAC Germicidal Spray Product Test is a carrier-based test (see ref. 15.4). Carriers (glass slides) are inoculated with a test organism, dried, exposed to the use-dilution of the disinfectant product, and cultured to assess the survival of the bacteria.

A single test with one use-dilution organism (*P. aeruginosa* or *S. aureus*) involves the evaluation of 60 inoculated carriers against one product sample. In addition to the 60 carriers, 6 carriers are required to estimate

carrier bacterial load and 6 more are included as extras. Thus, a total of 72 seeded carriers are required to perform a single test.

A single test (one product sample) with *M. bovis* (BCG) involves 15 total inoculated carriers (10 for testing, 3 for carrier counts, and 2 extras).

## 10.2 Test Culture Preparation for *P. aeruginosa* and *S. aureus*

- 10.2.1 Initiate test culture by inoculating a 10 mL tube (20 mm x 150 mm) of nutrient broth or synthetic broth from a stock slant culture. Transfer a loopful of inoculum from the stock slant into the broth. Refer to SOP MB-02, Test Microbes, for stock culture preparation.
- 10.2.2 Two sets of cultures of the same organism may be initiated in parallel from the same stock culture and subcultured; however, only one set of the final cultures is used for actual testing.
- 10.2.3 The test culture is serially subcultured for at least three consecutive  $24 \pm 2$  hour periods in 10 mL of nutrient broth or synthetic broth at  $37 \pm 1^\circ\text{C}$ .
- 10.2.4 The test culture is subcultured once again in nutrient broth or synthetic broth and incubated at  $37 \pm 1^\circ\text{C}$  for 48 to 54 hours. For this final subculture step, inoculate six to eight 25 mm × 150 mm tubes containing 20 mL of nutrient broth or synthetic broth for each test.
- 10.2.5 Reminder: The culture sequence must begin on Thursday for testing to commence on the following Tuesday.
- 10.2.6 Record all culture transfers on the Organism Culture Tracking form (see SOP MB-02, Test Microbes).
- 10.2.7 Pool the six to eight 48-54 hour test cultures in a sterile flask.
- 10.2.8 The pellicle from the 48-54 hr *Pseudomonas* cultures must be removed from the broth before pooling the culture either

by decanting the liquid culture aseptically into a sterile tube or by gently aspirating the broth culture away from the pellicle using a sterile 10 mL pipette. In either case the pellicle must not be broken or fragmented or the culture is not usable.

- 10.2.9 Swirl to mix.
- 10.2.10 If no organic soil is required, aliquot 20 mL portions of the culture into sterile 25 mm x 150 mm test tubes.
- 10.2.11 If an organic soil load is to be added to the culture, measure the pooled culture and add the appropriate amount of soil to the flask. Swirl to mix. Aliquot 20 mL portions into sterile 25 mm x 150 mm test tubes.
- 10.2.13 Vortex the 20 mL cultures for 3-4 sec and let stand 10 minutes at room temperature.
- 10.2.14 Withdraw the top 3/4 of the culture from each tube with a sterile pipette and dispense a total of 20 mL into sterile 25 mm x 150 mm test tubes. Cultures may be combined from more than one tube to achieve the 20 mL total. Prepare four tubes in this way. These cultures will be used to seed carriers.

### 10.3 Test Culture Preparation for *Mycobacterium bovis* BCG:

- 10.3.1 Generation of Cultures used in Testing. Each week (during testing), select 2-4 M7H9 stock cultures with typical growth and transfer a loopful of growth into the MPB broth tubes (20 mL in 25 mm X 150 mm tubes).
- 10.3.2 Inoculate 12 MPB broth tubes. Growth from 1 M7H9 slope can be used to inoculate multiple MPB tubes.
- 10.3.3 Incubate in a slanted position without disturbing for 21-25 days at  $37 \pm 1^{\circ}\text{C}$ .
- 10.3.4 Using the 12, 21-25 day old cultures grown in MPB medium,

inoculate approximately 20-40 25 mm X 150 mm tubes containing 20 mL of MPB. Incubate in a slanted position without disturbing for 21-25 days at  $37\pm 1^{\circ}\text{C}$ . Record all transfers on the Organism Culture Tracking Form.

- 10.3.5 Depending on the amount of growth from each 21-25 day old culture, 10-20 of the cultures may be required to generate enough standardized inoculum (approx. 75 mL) for a "typical" test day. A typical test day will require 30 seeded glass slide carriers, 1 carrier per petri dish. These carriers are used for the following:

Test of 2 product samples (30 total carriers; 20 for testing, 3 for carrier counts, 7 are extras).

The additional cultures are available in the event that growth in some tubes is weak.

- 10.3.6 On the day of the test, using a sterile transfer loop, carefully harvest the growth from the surface of the 20 mL cultures; transferring the growth collected from an individual 20 mL culture into a sterile glass tissue grinder.
- 10.3.7 Add 1 mL of 0.1% Tween 80 in saline solution to each glass tissue grinder. Homogenize the culture to break up large clumps or aggregates of bacteria.
- 10.3.8 Add 9 mL of MPB media to the homogenized culture.
- 10.3.9 Using a sterile pipette, transfer the homogenized *M. bovis* (BCG) suspension from the tissue grinder to a sterile test tube. Allow the culture to settle for 10-15 minutes to allow large clumps to settle out of suspension. Using a sterile pipette, transfer and pool the culture that remains in suspension to a clean, sterile flask.
- 10.3.10 Measure the transmittance of the pooled culture using the Beckman DU Series 500 spectrophotometer. Dilute the pooled culture with MPB medium until the culture gives 20.0% ( $\pm 1.0\%$ ) transmittance at 650 nm. Note: Wear

NIOSH certified respirators or face shields during this process.

- 10.3.11 If an organic soil load is specified in the test parameters for the product test, measure the culture and add the appropriate amount of soil to the flask. Swirl to mix.
- 10.3.12 Using a sterile 25 mL pipette, aseptically transfer 24 mL quantities of the culture into sterile 25 mm X 150 mm test tubes.

#### 10.4 Disinfectant Sample Preparation:

- 10.4.1 Prepare disinfectant samples aseptically according to the test parameters. Use of the product, contact time, temperature, diluent, organic soil, hard water, and neutralizer will be specified. Record test parameter information on the Test Information Sheet (see 16.3).
- 10.4.2 Follow chain-of-custody guidelines for disinfectant samples as stipulated in SOP COC-01, Chain-of-Custody.
- 10.4.3 For spray products which require preparation (e.g., preparing a use-dilution, use of a pump or trigger based sprayer) proceed as described in 10.4.4 through 10.4.8.
- 10.4.4 To ensure stability, prepare the disinfectant dilutions within three hours of performing the assay unless test parameters specify otherwise.
- 10.4.5 Prepare all dilutions with sterile standardized volumetric glassware. Record preparation of disinfectant on the Media and Reagent Prep Sheet.
- 10.4.6 Prior to opening the container, gently shake the container and thoroughly clean the area around the cap and spout with 70% ethanol. Allow the surface to dry. Remove the cap. Do not touch the inside surface of the cap. If present, carefully remove the seal attached to the top of the spout with cooled, flamed-sterilized instruments (i.e., razor blade,

forceps).

- 10.4.7 Pour an appropriate aliquot of the sample into a sterile beaker. Do not place a pipette or any other instrument inside the product container. Place the cap on the product container and secure tightly. From the beaker, dispense ready-to-use products directly into sterile medication tubes or initiate dilutions for diluted products.
- 10.4.8 Use  $\geq 1.0$  mL of sample disinfectant to prepare the use-dilution to be tested. Use v/v dilutions for liquid products and w/v dilutions for solids. Round to two decimal places toward a stronger product.
- 10.4.9 For aerosol spray products, shake the can 25 times prior to use, unless otherwise specified by the manufacturer. The cans are immobilized in the Spray Disinfectant Apparatus (see Attachment B) and the distance from the nozzle to the seeded carrier is measured to ensure the correct distance. Prior to testing, the spray nozzle is wiped with 70% ethanol and allowed to dry. Spray the product for 10-15 seconds prior to commencement of the test.

#### 10.5 Carrier Preparation and Inoculation:

- 10.5.1 Place two pieces of Whatman No. 2 filter paper (8.5 cm diameter) into each 15 mm  $\times$  100 mm glass petri dish. Place one 25 mm<sup>2</sup> glass slide on top of the filter paper in each petri dish. Carriers must be screened prior to use (see SOP MB-03, Screening Carriers).
- 10.5.2 Autoclave for 25 min. at 121°C with a 20 min. dry cycle.
- 10.5.3 Transfer 0.01 mL of the test culture with a sterile capillary pipet or Eppendorf pipette with sterilized tips onto 25 mm<sup>2</sup> sterile dry glass slide. Spread the inoculum uniformly over entire area immediately using a sterile 4 mm loop.
- 10.5.4 Repeat the operation a total of 72 times (60 carriers for testing, 6 for carrier counts and 6 extras) for *S. aureus* or *P.*

*aeruginosa* and 15 times (10 carriers for testing, 3 for carrier counts, and 2 extras) for confirmatory tuberculocidal testing with *M. bovis* (BCG). After completion of the inoculation, thoroughly wipe the Eppendorf Pipette with 70% ethanol prior to removal from the BSC.

10.5.5 Dry the slides for 30 min. at  $37\pm 1^{\circ}\text{C}$  when performing tuberculocidal testing with *M. bovis* (BCG). Dry the slides for 40 min. at  $37\pm 1^{\circ}\text{C}$  for testing of *S. aureus* or *P. aeruginosa*.

10.5.6 Record timed carrier inoculation activities on the Time Recording Sheet for Inoculation (see 16.1).

10.6 General Test Procedure:

10.6.1 Once the container is immobilized in the apparatus, measure the distance from the nozzle tip to a sample petri dish containing an unseeded carrier. Make any necessary adjustments to ensure that this distance is within the range recommended on the test parameters.

10.6.2 Spray the disinfectant for a few sec. to clear any debris from the nozzle area and to ensure that the nozzle is not clogged.

10.6.3 Seeded slides are sprayed from the recommended distance for the prescribed period of time at 30 sec. intervals for *S. aureus* or *P. aeruginosa*, and at 1 min. intervals for confirmatory tuberculocidal testing with *M. bovis* (BCG).

10.6.5 The bacterial carrier load is assayed as stipulated in SOP MB-04, Carrier Counts.

10.7 Spray Tests with *S. aureus* or *P. aeruginosa*:

10.7.1 After the required drying time, the slides are sprayed at 30 sec. intervals while in the petri dish.

10.7.2 If a specific time is stipulated by the manufacturer other than a 10 min. exposure time, the interval is modified to

accommodate their claims.

- 10.7.3 The slide must be sprayed within  $\pm 5$  sec. of the specified time.
- 10.7.4 After the last slide of a set of slides (20 slides) has been sprayed with the disinfectant, and the exposure time is complete, transfer each slide in order, into the primary subculture tubes containing a neutralizer within the  $\pm 5$  seconds time limit. Transfers will be made with flame sterilized forceps.
- 10.7.6 Prior to transfer, drain the excess disinfectant from each slide. Transfer the slides in their corresponding subculture tubes at the appropriate time. The remaining slides are moved into their corresponding subculture tubes at the appropriate time.
- 10.7.7 The slide can touch both the interior sides of the petri dish and the subculture tube during the transfer, but this contact should be avoided as much as possible.
- 10.7.8 After the slide is deposited, the subculture tube is recapped and shaken for a few seconds. Alternately, a set of tubes may be shaken after all primary transfers are completed.
- 10.7.9 After all the slides have been transferred, the subculture tubes are placed in a  $37 \pm 1^\circ\text{C}$  incubator.
- 10.7.10 A minimum of 30 minutes after the last slide was deposited, transfer each slide to a secondary subculture tube containing 20 mL of the appropriate subculture media.
- 10.7.11 Move the slides in order but the movements do not have to be timed. Shake the tubes after all of the slides have been transferred.
- 10.7.12 Incubate the primary and secondary subculture tubes at  $37^\circ\text{C}$  for  $48 \pm 2$  hr. A total of 120 tubes will be incubated per sample tested per organism.

- 10.7.13 See Attachment A (Testing Footnotes and Explanations) for a list of footnotes which are used to indicate problematic events or observations which occurred during testing.

10.8 Results for *P. aeruginosa* and *S. aureus*:

- 10.8.1 Report results as + (growth) or 0 (no growth) on the Germicidal Spray Test: Germicidal Spray Test Results Sheet (see 16.4).
- 10.8.2 Each tube is shaken prior to recording results to determine the presence or absence of turbidity. A positive result is one in which the broth culture appears turbid. A negative result is one in which the broth appears clear.
- 10.8.3 A positive result in either the primary or secondary subculture tube is considered a positive result for a carrier set.

10.9 Confirmation Procedures for Spray Tests with *P. aeruginosa* and *S. aureus*:

- 10.9.1 If available, a minimum of three positive carrier sets (set = 1 primary and 1 secondary tube), should be confirmed using gram staining, selective media, and VITEK or API analysis.
- 10.9.2 If there are fewer than three positive carrier sets, then each carrier set will be confirmed. If both tubes are positive in a carrier set, only one tube is selected for confirmation.
- 10.9.3 For a test with greater than 20 positive carrier sets, Gram stain at least 20%.
- 10.9.3.1 Confirm a minimum of 4 positive carrier sets by Gram staining, selective media, and API or VITEK analysis. If both tubes are positive in a carrier set, only one tube is selected for confirmation.
- 10.9.4 Gram stain reactions, cell morphology, and colony

characteristics on selective media are given in SOP MB-02, Test Microbes.

- 10.9.5 Gram stains are performed on smears taken from the positive culture tubes.
- 10.9.6 For the additional confirmatory tests, a loopful of broth from each selected culture tube is streaked on both TSA and selective media appropriate for the test organism and incubated for  $24 \pm 2$  hr at  $37 \pm 1^\circ\text{C}$ .
- 10.9.7 The selective agar is checked for the correct reaction and the culture on the TSA plate is used for preparing the inoculum for the API strips or VITEK unit.
- 10.9.8 The VITEK or API test should be performed according to the manufacturer's instructions (see ref. 15.1, 15.2 and 15.3).
- 10.9.9 If confirmatory testing determines that the identity of the organism was not the test organism, the positive entry (+) on the results sheet must be annotated to indicate a contaminant was present. A footnote of "C" will be applied to the entry to indicate that the growth was determined not to be the test microbe (see Attachment A for list of footnotes).

#### 10.10 Spray Tests with *M. bovis* (BCG):

- 10.10.1 After the required drying time in the petri dish, slides are sprayed at 60 second intervals.
- 10.10.2 If a specific time is stipulated by the manufacturer other than a 10 min. exposure time, the interval is modified to accommodate their claims.
- 10.10.3 The slide must be sprayed within  $\pm 5$  sec. of the prescribed time.
- 10.10.4 After one set of slides has been sprayed with the disinfectant, and the exposure time is complete, the slides

are then transferred in the same sequentially timed fashion into the neutralizer tubes containing the appropriate neutralizer (*e.g.*, letheen neutralizer blank or horse serum).

- 10.10.5 The slide is removed from the petri dish with flame sterilized forceps.
- 10.10.6 Prior to transfer, drain the glass slide to remove the excess disinfectant. Transfer the carrier into a tube of neutralizer.
- 10.10.7 As with the transfers to the tubes containing the disinfectant, primary transfers should be within  $\pm 5$  sec. of the specified time of transfer.
- 10.10.8 The neutralizer tube is shaken and the slide is immediately removed from the neutralizer tube with flame sterilized forceps and transferred to MPB or the designated subculture tube.
- 10.10.9 The remaining slides are moved into their corresponding neutralizer and subculture tubes at the appropriate times.
- 10.10.10 After the slide is deposited, the subculture tube is recapped and shaken for a few seconds. Alternately, the tubes may be shaken after all primary transfers are completed. The lip of the subculture tube does not need to be flamed.
- 10.10.11 Once all slides have been transferred to the MPB medium, transfer 2 mL aliquots from the neutralizer tube into each of 2 tubes of the specified additional subculture media (Middlebrook 7H9 Broth, Kirchners Medium, TB Broth); repeat with all ten slides. That is, two tubes each of two additional subculture media receive 2 mL of neutralizer for a total of 4 tubes of additional subculture media.
- 10.10.12 The transfers of neutralizer medium into subculture media are not timed.
- 10.10.13 Incubate all tubes for 60 days at  $37 \pm 1^\circ\text{C}$ . If no growth or occasional (insufficient for confirmation purposes) growth

occurs, incubate an additional 30 days before recording final results.

10.11 Recording Results for *M. bovis* (BCG):

- 10.11.1 Results are recorded as positive (+) or negative (0) as indicated by the presence or absence of growth. Prior to entering (+) or (0), an acid fast stain is performed (see 10.13).

10.12 Confirmatory Procedures for Spray Tests with *M. bovis* (BCG):

- 10.12.1 To confirm the results of testing, representative positive subculture tubes are selected for further investigation.
- 10.12.2 The maximum number of tubes that is confirmed per sample tested is 10.
- 10.12.3 At least one positive subculture tube for each carrier set with growth is confirmed.
- 10.12.4 If more than one subculture tube for a carrier set is positive, only growth in one subculture tube is confirmed. If the MPB in the set is positive, it is the representative subculture tube used for confirmation.
- 10.12.5 If MPB is not positive, then the order of selecting the representative subculture tubes for confirmation is: M7H9, Kirchners, and TB.
- 10.12.6 If growth is observed in only one carrier set (5 tubes per set), then all subculture tubes showing growth for that carrier are subject to confirmation.

10.13 Identification of *M. bovis* (BCG):

- 10.13.1 The confirmatory tests used to verify the identity of *M. bovis* (BCG) are acid fast staining and plating on selective media.
- 10.13.2 A smear for acid fast staining is taken from the selected

tubes with growth on the day that results are read. Acid fast rods are typical for *M. bovis* (BCG). The acid fast staining results should be read promptly and prior to assigning a (+) or (0) to the results.

- 10.13.3 If acid fast rods are observed then a (+) is assigned to the results. If no cells are observed for the acid fast stain then a (0) is applied to the results.
- 10.13.4 In addition, growth from these positive tubes is struck over the surface of a Middlebrook 7H9 (M7H9) agar plate, a selective medium, and incubated for 21-25 days at  $37 \pm 1^{\circ}\text{C}$ .
- 10.13.5 If a satisfactory smear cannot be obtained directly from the tube, the smear for acid fast staining will be taken from the 21-25 day old M7H9 agar plate that was inoculated with the growth from the tube.
- 10.13.6 In the event that no cells were observed with acid fast staining initially but typical growth was observed on the M7H9, then the (0) will be corrected to read (+) on the test sheet. An entry error will be noted in the comments section of the results sheet (16.6).
- 10.13.7 Following the 21-25 day incubation period, the colony morphology of the organism on M7H9 agar is evaluated. *M. bovis* (BCG) typically appears as opaque to buff-colored, raised, rough growth on M7H9 agar. Refer to Table 10.3 in SOP MB-02, Test Microbes, for a detailed description of *M. bovis* (BCG).
- 10.13.8 Record confirmation results on the Test Microbe Confirmation Sheet (see 16.7).

11.0 DATA ANALYSIS/CALCULATIONS: None

12.0 DATA MANAGEMENT/RECORDS MANAGEMENT:

- 12.1 Data will be recorded promptly, legibly, and in indelible ink on the appropriate forms (see 16.0). Completed forms are archived in notebooks

kept in locked file cabinets adjacent to offices D217. Only authorized personnel have access to the locked files. Archived data is subject to OPP's official retention schedule contained in SOP ADM-01, Records and Archives.

13.0 QUALITY CONTROL:

13.1 The OPP Microbiology Laboratory conforms to 40 CFR Part 160, Good Laboratory Practices. Appropriate quality control measures are integrated into each SOP.

13.2 For quality control purposes, the required information is documented on the appropriate forms (see 16.0)

14.0 NONCONFORMANCE AND CORRECTIVE ACTION:

14.1 Strict adherence to the protocol is necessary for the validity of the test results. Any deviation from the standard protocol must be brought to the study director's attention and recorded in the raw data and an explanation for the deviation given. The deviation and reason for it must be documented on the GLP Compliance form in the final report.

15.0 REFERENCES:

15.1 bioMérieux. 1995. Industrial VITEK Reference Manual No. 510713-1, Revision Date 07-1995. bioMérieux VITEK, Inc., Hazelwood, MO.

15.2 bioMérieux S.A. 1997. Analytical Profile Index Reference Book Number 20 090 (20 NE), 6<sup>th</sup> Edition. Marcy-I'Etoile, France.

15.3 bioMérieux S.A. 1997. Analytical Profile Index Reference Book Number 20 590 (Staph), 4<sup>th</sup> Edition. Marcy-I'Etoile, France.

15.4 Official Methods of Analysis. 1990. 15<sup>th</sup> Ed., Association of Official Analytical Chemists, Arlington, VA, (Method 961.02).

16.0 FORMS AND DATA SHEETS:

16.1 Germicidal Spray Test: Time Recording Sheet for Carrier Inoculation Steps

- 16.2 Germicidal Spray Test: Time Recording Sheet for Carrier Transfers
- 16.3 Germicidal Spray Test Information Sheet
- 16.4 Germicidal Spray Test Results Sheet
- 16.5 AOAC Germicidal Spray Test Information Sheet For *M. bovis* (BCG)
- 16.6 Germicidal Spray Test Results Sheet for *M. bovis* (BCG)
- 16.7 Test Microbe Confirmation Sheet

Attachment A: Testing Footnotes

Attachment B: Spray Apparatus

# AOAC Germicidal Spray Test: Time Recording Sheet for Carrier Inoculation Steps OPP Microbiology Laboratory

TEST INFORMATION/Confirmed by:_____	
Test Date	
Type of Test	
Product Reg. No.	
Product Name	
Sample No(s).	

Initials/Date	Test ID	Inoculum Settle Time*		Carrier Seeding Time*		Carrier Dry Time*	
		Start Time	End Time	Start Time	End Time	Start Time	End Time
		/	/	/	/	/	/
		/	/	/	/	/	/
		/	/	/	/	/	/
		/	/	/	/	/	/
		/	/	/	/	/	/
		/	/	/	/	/	/
Comments:							

\* Recorded from laboratory clock/and timer.

# AOAC Germicidal Spray Test: Time Recording Sheet for Carrier Transfers OPP Microbiology Laboratory

TEST INFORMATION/Confirmed by:_____	
Test Date	
Type of Test	
Product Reg. No.	
Product Name	
Sample No(s).	
Test Organism	

Initials/date	Set	Drop Interval	Carrier Spray Start Time (into the disinfectant)		Carrier Spray End Time (into the neutralizer/primary subculture) <sup>1</sup>		Carrier Transfer (into secondary subculture)
			Clock	Timer	Clock	Timer	Start Time <sup>2</sup>
	1-20						
	21-40						
	41-60						
Comments:							

<sup>1</sup> For spray test with *M. bovis*, the slide end time is when the slide is transferred into the neutralizer tube. The slide is then immediately transferred into MPB.

<sup>2</sup> Carrier transfer into secondary subculture; taken from clock

## AOAC Germicidal Spray Test Information Sheet

### OPP Microbiology Laboratory

TEST INFORMATION/Confirmed by: _____			
EPA Reg. No.		SOP	
Name		Test Date	
Sample No.		Comments/Modifications:	
Lot No.			

TEST PARAMETERS/Confirmed by: _____				
H <sub>2</sub> O Hardness (CaCO <sub>3</sub> ) ppm	Specified	Titrated (Buret)/Date/Init		HACH/Date/Init
		/ /		/ /
Use Dilution	Specified	As Prepared/Date/Init		
		/ /		
Organic Soil	Specified	As Prepared/Date/Init		
		/ /		
Neutralizer	Specified			
Temperature	Specified	Chiller Unit Display		Test Tube Waterbath
		Before:	After:	Before: After:
Contact Time	Specified	As Tested		
Other Parameters	Specified			

TEST MICROBE INFORMATION/Confirmed by: _____				
Test Microbe		48-54 Hour Culture		
Org. Control No.		Date/Time	Initiated	Harvested
Avg. CFU/Carrier				

REAGENT/MEDIA INFORMATION/Confirmed by: _____			
Reagent/Media	Prep. No.	Reagent/Media	Prep. No.

## Germicidal Spray Test Results Sheet

OPP Microbiology Laboratory

TEST INFORMATION/Confirmed by:_____			
EPA Reg. No.		Test Date	
Name		Test Organism	

CARRIER INFORMATION/Confirmed by:_____		
Carrier Spray Time Interval	Carrier Set	Analyst

TEST RESULTS									
Date/Initials									
Primary Subculture / Secondary Subculture (carrier)									
1	2	3	4	5	6	7	8	9	10
/	/	/	/	/	/	/	/	/	/
11	12	13	14	15	16	17	18	19	20
/	/	/	/	/	/	/	/	/	/
21	22	23	24	25	26	27	28	29	30
/	/	/	/	/	/	/	/	/	/
31	32	33	34	35	36	37	38	39	40
/	/	/	/	/	/	/	/	/	/
41	42	43	44	45	46	47	48	49	50
/	/	/	/	/	/	/	/	/	/
51	52	53	54	55	56	57	58	59	60
/	/	/	/	/	/	/	/	/	/
Comments:									

# AOAC Germicidal Spray Test Information Sheet For *M. bovis* (BCG) OPP Microbiology Laboratory

TEST INFORMATION/Confirmed by: _____			
EPA Reg. No.		SOP	
Name		Test Date	
Sample No.		Comments:	
Lot No.			

TEST PARAMETERS/Confirmed by: _____				
H <sub>2</sub> O Hardness (CaCO <sub>3</sub> ) ppm	Specified	Titrated (Buret)/Date/Init		HACH/Date/Init
		/ /		/ /
Use Dilution	Specified	As Prepared/Date/Initials		
		/ /		
Organic Soil	Specified	As Prepared/Date/Init		
		/ /		
Neutralizer	Specified			
Temperature	Specified	Chiller Unit Display		Test Tube Waterbath
		Before:	After:	Before: After:
Contact Time	Specified	As Tested		
Other Parameters	Specified			

TEST MICROBE INFORMATION/Confirmed by: _____				
Test Microbe		21-25 Day Culture		
Org. Control No.		Date/Time	Initiated	Harvested
Avg. CFU/Carrier				

REAGENT/MEDIA INFORMATION/Confirmed by: _____			
Reagent/Media	Prep. No.	Reagent/Media	Prep. No.

Germicidal Spray Test Results Sheet for *M. bovis* (BCG)  
OPP Microbiology Laboratory

TEST INFORMATION/Confirmed by:_____			
EPA Reg. No.		Test Date	
Name		Test Organism	

CARRIER SPRAY/NEUTRALIZER TRANSFER INTO SECONDARY SUBCULTURE INFORMATION/Confirmed by:_____	
Step	Analyst Performing Step
Carrier Spray Interval:_____	
Neut. Transfer	
Neut. Transfer	
Neut. Transfer	

TEST RESULTS										
Date Recorded/Initials				60 Day:_____/90 Day:_____						
60 Day Results/90 Day Results										
Media	Carrier									
	1	2	3	4	5	6	7	8	9	10
MPB	/	/	/	/	/	/	/	/	/	/
M7H9 <sup>1</sup>	/	/	/	/	/	/	/	/	/	/
	/	/	/	/	/	/	/	/	/	/
Kirchners <sup>1</sup>	/	/	/	/	/	/	/	/	/	/
	/	/	/	/	/	/	/	/	/	/
TB Broth <sup>1</sup>	/	/	/	/	/	/	/	/	/	/
	/	/	/	/	/	/	/	/	/	/
Comments:										

<sup>1</sup> There are two subculture sets for this media. The upper row represents subculture set one and the lower row subculture set two.

# Test Microbe Confirmation Sheet OPP Microbiology Laboratory

TEST INFORMATION/Confirmed by:_____			
EPA Reg. No.		Test Date	
Name		Test Organism	

Source: Tube/Plate ID	Date/ Initials	Stain Results *	Media Information			Results		
			Name	Prep. No.	Inc. Time/ Temp.	Date/ Initials	Colony Characteristics	API Test/VITEK ID** (if applicable)

\* GPC=gram positive cocci, GNR=gram negative rods, AFR=acid fast rods

\*\* API or VITEK numerical profile number

\*\*\* Use MRME notation for all organisms except *M. bovis*; use MR notation for *M. bovis* (BCG).

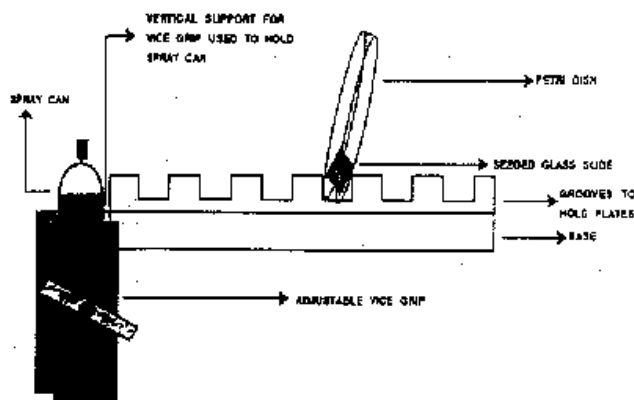
## Attachment A:

### Testing Footnotes and Explanations OPP Microbiology Laboratory

Footnote	
A	Indicates that the seeded carrier, hook, or forceps hit the interior sides of the medication tube containing disinfectant as the carrier was being dropped.
B	Indicates that the carrier was lost (dropped) during a transfer and was not recovered.
C	Indicates that a tube of a positive carrier set (one showing growth) was later determined to be a contaminant and not the test microbe. In "Comments" refer to the confirmation information for details.
D	Indicates that the primary or secondary subculture tube containing the carrier broke during vortexing. In the "Comments" indicate if carrier was recovered or if the remaining broth was placed in another tube.
E	Indicates that the carrier was exposed to the disinfectant late or early, outside of the +/- 5 second drop, spray, or wipe interval. In "Comments" indicate the approximate number of seconds outside (+/-) of the 5 second interval.
	Indicates that the carrier was placed in the neutralizer late or early, outside of the +/- 5 second drop interval. In "Comments" indicate the approximate number of seconds outside (+/-) of the 5 second interval.

Attachment B:

SIDE VIEW OF SPRAY CAN HOLDER USED  
FOR SPRAY DISINFECTANT TEST



FRONT VIEW OF SPRAY CAN HOLDER USED  
FOR SPRAY DISINFECTANT TEST

